Malays J Pathol 2025; 47(1) : 113 – 120

BRIEF COMMUNICATION

In Vitro multinucleated giant cell–like cells formation: An observation

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Abstract

Introduction: The formation of multinucleated giant cells (MGCs), is known to occur during chronic inflammations. However, the molecular mechanisms and cell signalling pathways by which they adhere are largely unknown. Most studies concerning the development of MGCs involve the stimulation of isolated monocytes or macrophages with infectious pathogens. However, such *in vitro* studies are limited, and the resulting MGCs do not accurately mimic MGC observed *in vivo*. In this report, we discuss an observation of the emergence of MGCs derived from umbilical cord blood in a modified methylcellulose culture. *Materials and Methods:* A 10-day colony-forming unit (CFU) assay (methylcellulose culture) using umbilical cord blood mononuclear cells resulted in the emergence of adherent epithelioid-like clusters. *Results:* During the latter stages of the culture timeline, monocytic-like cells were observed to emerge from within these adherent clusters, alongside the formation of large MGCs exhibiting their characteristic morphology of multiple nuclei. *Conclusions:* This observation could serve as a reference for cell culture studies and may present an alternative model of monocytic-lineage cell development, involving an intermediary stage rather than the conventional linear haematopoietic hierarchy of blood cell differentiation.

Keywords: Multinucleated Giant Cells, Macrophage, Haematopoiesis

INTRODUCTION

Multinucleated giant cells (MGCs) are morphologically defined as cells containing one or more nuclei and phagocytic vacuoles within a shared cytoplasm with individual MGCs exceeding 100 μm lengthwise.[1,2] They may arise as part of the body's immune response to an infectious disease such as tuberculosis (forming Langhans' giant cells, LHCs), or through the fusion of multiple macrophages encapsulating a foreign substance (forming foreign body giant cells, FBGCs).[2,8]

MGCs belong to the myeloid cell lineage, often described in the literature as the fusion of multiple macrophages.[1] For a long time, it was generally accepted that macrophage formation follows the haematopoiesis model whereby beginning in the bone marrow, myeloid progenitor cells give rise to circulating mature monocytes before differentiating into macrophages during inflammation.[10] However, there have been reports of macrophages developing through embryonic haematopoiesis in the yolk sac, with minimal derivation from monocytes.[3] These reports suggest that mechanisms of MGC formation may not strictly adhere to the more widely accepted model of linear development.

Attempts to culture MGCs *in vitro* have largely been limited, with most studies focusing on the cytokine stimulation of isolated monocytic cells and their biochemical surface interactions. However, investigations into the biology of one type of giant cell do not necessarily translate well into other types of giant cells. Additionally, the generation of MGCs reported in the literature may not necessarily reflect those in actual physiological conditions due to the excessive cell manipulation required for stimulation using concentrated cytokines.

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In this report, we present an observation of MGC-like cell formation derived from umbilical cord blood mononuclear cells cultured in a commercial methylcellulose medium. The intended objective of the original study was to generate monocytic lineage precursor cells using a methylcellulose medium, but in doing so we observed an abnormal formation of adherent cell clusters and MGC-like cells. We hypothesised that a prior infection experienced by the cord blood sample donor may have caused the pre-stimulation of precursor cells, resulting in cells that were predisposed to differentiate into intermediary adherent epithelioid-like cells and subsequently form MGC-like cells.

MATERIALS AND METHODS

Cell culture

Cord blood mononuclear cells (CB MNCs) were obtained from Cryocord Sdn. Bhd. CB MNCs were seeded at a density of 1 × 10^5 cells/ml in MethoCult™ H4535 Enriched Without EPO (STEMCELL Technologies, Canada) according to manufacturer's protocol, and incubated at 37°C for 10 days.

Characterisation

Morphological analysis was conducted using phase-contrast microscopy with a NIKON Eclipse Ts2 inverted microscope (Nikon, USA) to monitor colony growth patterns throughout the entire culture period. MGC-like morphology observed in the cultures was verified by a haematologist.

For flow-cytometric analysis, cells were washed once upon harvest with 1× PBS, counted, and adjusted to a cell concentration of about 1 × 10^6 cells/mL in 5 mL round bottom PP tubes (Becton Dickinson, USA), designated as 'Unstained', 'Primitive', and 'Mature'. The 'Unstainned' tube served as a negative control and contained cells that were not incubated with any fluorochrome-conjugated antibodies. Subsequently, the cells were incubated with fluorochrome-conjugated monoclonal antibodies targeting the markers of interest at 4 °C for 30 minutes in the dark according to the following panel as illustrated in Table 1.

Following incubation, cells were washed twice with 0.2% BSA/1× PBS before acquisition using FACSCanto™ II flow cytometer (Becton

TABLE 1: Panel of fluorochrome-conjugated monoclonal antibodies used for flow cytometric analysis

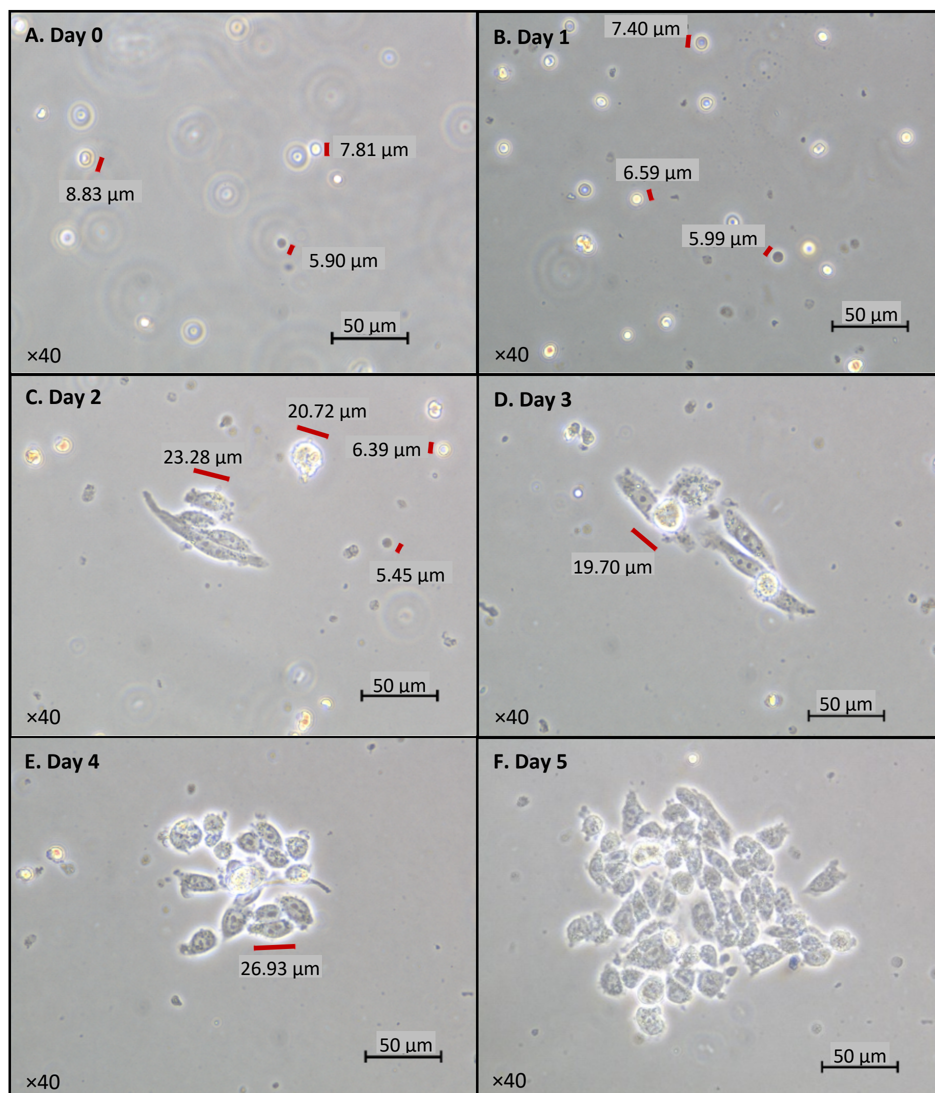
Panel	Marker	Fluorochrome	Antibody Description	Manufacturer	Catalog No.
Primitive	CD34	PE	Mouse Anti-human	BD Biosciences, USA	MAB348057
	CD117	PE-Cy7	Mouse Anti-human	BD Biosciences, USA	MAB339195
	CD133	APC	Mouse Anti-human	R&D Systems, USA	GZ-FA-B11331A-025
	CD38	APC-H7	Mouse Anti-human	BD Biosciences, USA	PMG656646
Mature	CD34	PE	Mouse Anti-human	BD Biosciences, USA	MAB348057
	CD117	PE-Cy7	Mouse Anti-human	BD Biosciences, USA	MAB339195
	CD11c	PerCP-Cy5.5	Mouse Anti-human	BD Biosciences, USA	PMG658330
	CD14	FITC	Mouse Anti-human	BD Biosciences, USA	MAB347493
	CD64	APC	Mouse Anti-human	BD Biosciences, USA	PMG561189
	CD4	APC-H7	Mouse Anti-human	BD Biosciences, USA	MAB641398

Dickinson, USA) equipped with red and blue lasers. Data analysis was performed using FACS DIVA software. Gating strategies included initial exclusion of debris based on forward scatter (FSC) and side scatter (SSC), followed by doublet discrimination and exclusion of dead cells. Subsequent gates were defined based on fluorochrome-specific fluorescence intensity to delineate subpopulations of interest.

RESULTS

On Day 0, the freshly inoculated cells appeared small and spherical, typically measuring less than 10 µm in length, and they retained a similar morphology on Day 1 (Figure 1A–B). Adherent clusters of epithelioid-like cells were observed to have formed beginning on Day 2 and by Day 9 MGC-like cells were observed (Figure 1C–K). MGC-like cells presented as irregularly shaped large flat discs, often exceeding 100 µm lengthwise. They possessed multiple nuclei within a shared cytoplasm but MGC-like cells having a single nucleus were also observed. Accompanying these characteristics was the presence of phagocytic vacuoles dotting around the cytoplasm.

To further investigate the occurrence of these aberrant formation of adherent cells and MGC-like cells from what seemed like normal cord blood MNCs (Figure 2) and haematopoietic stem cells (HSCs), we then compared the white blood cell count (WBC) data between two donated samples. The following Table 2 showed that the aberrant sample had a 2.9× higher WBC count



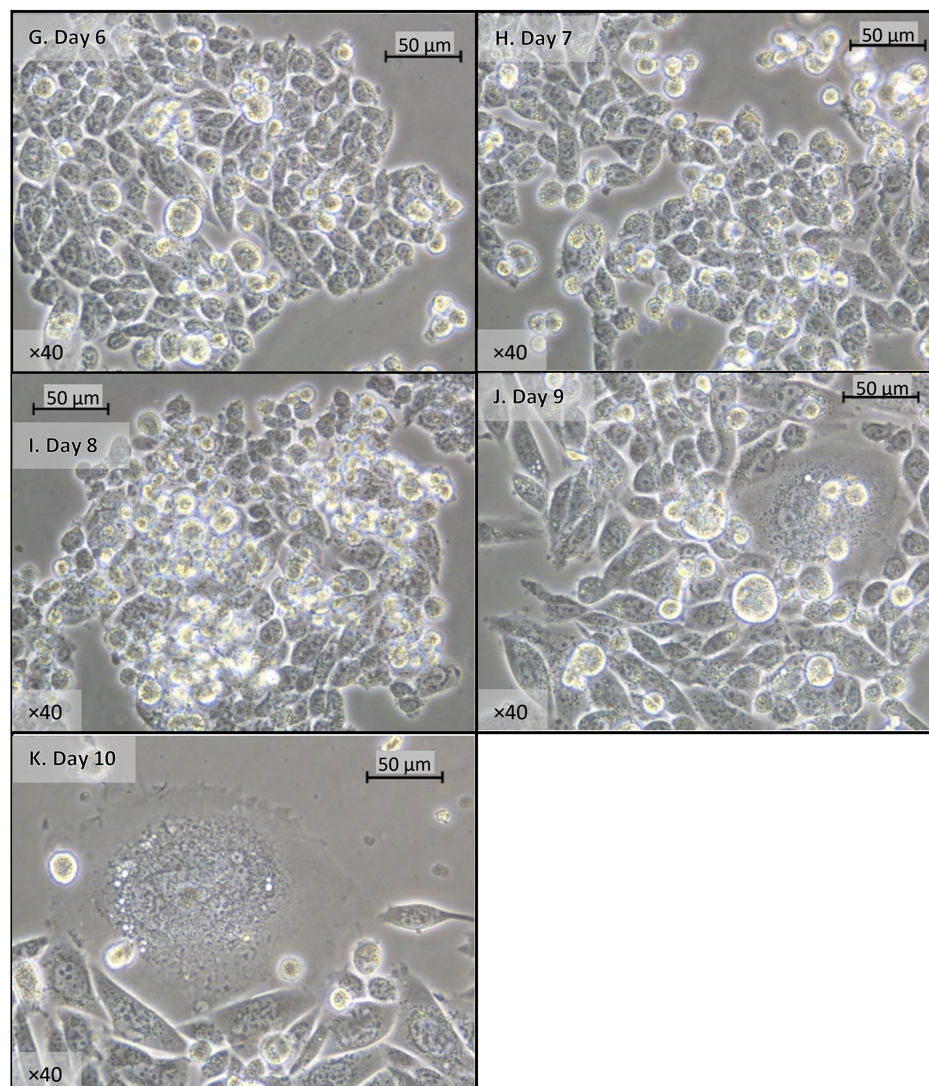


FIG 1. Phase-contrast microscopy of the growth profile of cord blood mononuclear cells (CB MNCs) in Methocult over 10 days. A) Freshly inoculated CB MNC; B) Day 1 CB MNC observed as suspended single cells; C) Small clusters of adherent cells were observed at bottom of culture plate; D-F) Continuous expansion of clusters; G-I) Non-adherent monocyte-like cells were observed to emerge from the adherent clusters; J-K) Large multinucleated giant cell (MGC)-like cell with multiple nuclei.

Table 2. Cell count data comparison between normal and aberrant samples. The aberrant sample showed a much higher white blood cell count than the normal sample

Abbr.	Description	Normal		Aberrant		Limits	
WBC	White blood cell count	17.9×10^9/L	H	52.2×10^9/L	H	**4.5**	**10.5**
LY	Lymphocyte count	-		56.9 %	H	**20.5**	**51.1**
MO	Monocyte count	-		22.9 %	H	**1.7**	**9.3**
GR	Granulocyte count	9.5 %	L	20.2 %	L	**42.2**	**75.2**
LY#	Lymphocyte count	-		29.7×10^9/L	H	**1.2**	**3.4**
MO#	Monocyte count	-		12.0×10^9/L	H	**0.1**	**0.6**
GR#	Granulocyte count	1.7×10^9/L		10.5×10^9/L	H	**1.4**	**6.5**
RBC	Red blood cell count	0.17×10^6/uL	L	0.18×10^6/uL	L	**4.00**	**6.00**

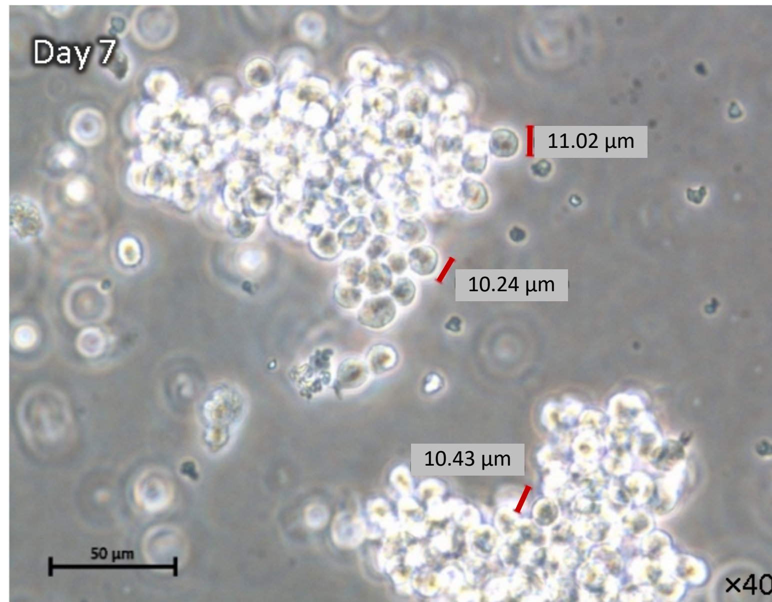


FIG 2. A culture of normal cord blood mononuclear cells (MNCs) on Day 7 in MethoCult, showing typical haematopoietic precursor cell cluster formation. The individual cells were small and spherical, with diameters ranging from 10-12 µm. No adherent cells or other cell types were observed in this culture.

than the normal sample, or approximately 5× the upper limits of normal blood count range, suggesting that the donor may have experienced a prior infection which had activated their physiological inflammatory response, which then predisposing their immune cell development in a particular way.

We then performed comparative flow cytometry analysis (Figure 3) on the starting materials of the aberrant cord blood sample and normal cord blood sample.

Flow-cytometry analysis of primitive marker CD133 (indicating cell immaturity) showed that the aberrant and normal samples expressed

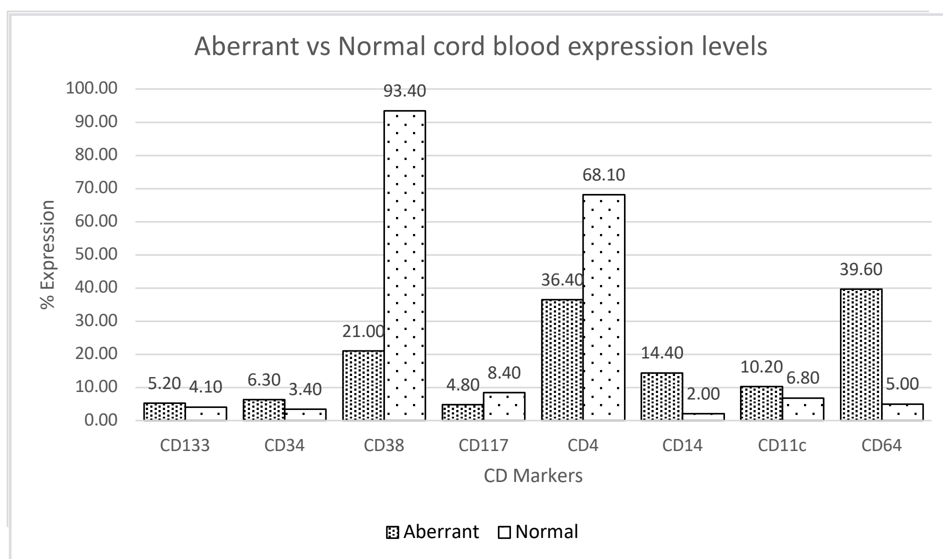


FIG 3. Comparing CD markers % expression of aberrant vs normal cord blood samples. Both samples showed similar primitive levels. However aberrant sample showed higher levels of monocytic markers (i.e. CD14 at 34.40%; CD64 at 39.60%) compared to the normal sample (i.e. CD14 at 2.00%; CD64 at 5.00%), potentially indicating lineage commitment.

similarly low levels (aberrant sample at 5.20%; normal sample at 4.10%). For CD34 (indicative of hematopoietic-lineage cells), the aberrant sample (6.30%) expressed slightly higher levels than of the normal sample. (3.40%). However, they differed in more mature markers such as the epithelioid-potential associated marker CD38, with the aberrant sample expression at 21.00%, and normal sample at 93.40% (higher expression levels in the normal sample were expected as this correlates with known literature where CD38 is a primitive pan myeloid marker with HSC potential), and monocytic markers CD14 (aberrant sample at 14.40%; normal sample at 2.00%) and CD64 (aberrant sample at 39.60%; normal sample at 5.00%), suggesting that the aberrant cells were likely already in a lineage-driven transitional phase, having been pre-stimulated.

DISCUSSION

In our observations, it appears that for MGC-like cells to develop there needs to be a prior stimulation or infection to have occurred at the progenitor stage, evidenced in part by the white blood cell count data showing that the aberrant sample was 2.9× more than the normal sample.

A potential method of MGC formation could be as a response mechanism to mycobacterial infection. A study by Lösslein *et al*. demonstrated that common monocyte progenitors (cMoP) in mice exhibited a high ability to form MGC in response to mycobacterial glycolipid lipomannan introduction, with cholesterol and lipid accumulation in the cytosol identified as a prerequisite to MGC formation.[7] Similarly in another study, Kawamura *et al*. identified a human cMoP analogue as a CLEC12A^{high}CD64^{high} subset in the GMP fraction of umbilical cord blood and bone marrow, suggesting similar MGCs transformation pathways in humans via mycobacterial infection.[6]

However, the development of MGCs via an intermediary epithelioid-like cell stage as shown in our study challenges the conventional methods of MGCs formation. For example, most studies involved the stimulation (via cytokines or bacterial components) of isolated monocytic progenitor cells or monocytes to induce MGC formation, often via the fusion of multiple macrophages post-stimulation.[9]

In our study, at the time of cell inoculation, morphological and flow-cytometry analyses indicated that no mature immune cells were detected in the starting material, evidenced by the flow-cytometry data showing little to no expression of CD markers indicating cell maturity. This observation suggests that the epithelioid-like adherent cells and their MGC-like cell progeny were not descended from mature cell types being stimulated by cytokines in the culture medium.

Phase-contrast microscopy revealed that the MGC-like cells observed in our cultures (Figure 4A) and the polymorphonuclear multinucleated cells spontaneously formed from secondary mouse fibroblasts as observed in a study by Holt and Grainger (Figure 4B) shared similar morphological features. Despite their different origins, both cell types displayed cytoplasmic spreading, and exhibited a comparable multinucleated structure. These

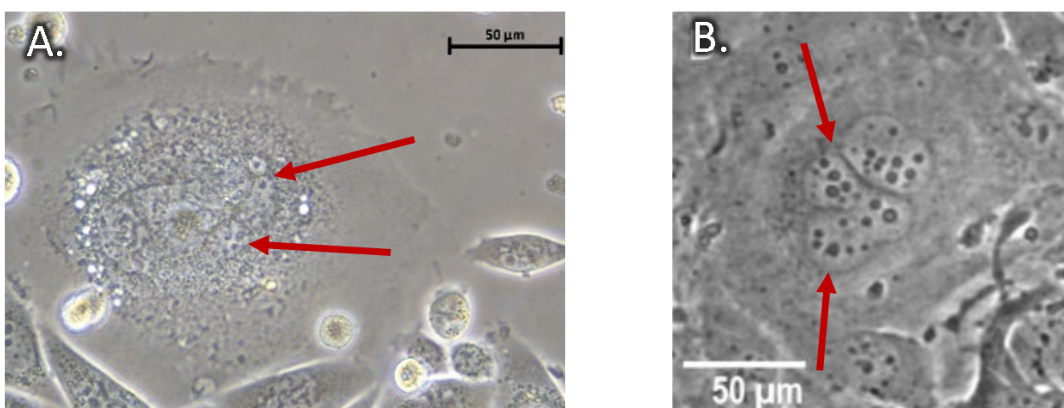


FIG 4. MGC-like cells observed in our cultures; A) Polymorphonuclear multinucleated cells with multiple nuclei, spontaneously formed from secondary mouse fibroblasts; B) Image adapted from Holt and Grainger.[4] The morphological similarities between them suggest that both could be of the same cell type.

similarities suggest that they may be of the same cell type.

As blood cells must transition through an epithelioid-associated phase (CD38 epithelioid potential), its presence in our starting sample, alongside immunity-associated markers CD14 and CD64, suggests that the aberrant sample cells may be a cell type with an intermediary monocytic predisposition. This predisposition appears to result in the differentiation of adherent cells, which subsequently produce MGC-like cells. In summary, we postulate that the cell sample donor may have experienced a prior infection event, and the effects disrupted normal haematopoietic progenitor cell development pathways, resulting in the abnormal formation of MGC-like cells.

The method of MGC-like cell formation observed in our study warrants further investigation as it may present an alternative model of monocytic-lineage cell development through an intermediary stage, rather than following the commonly accepted linear haematopoietic hierarchy of blood cell differentiation. The MGC-like cells observed in our study were verified to closely resemble true MGCs by a renowned haematologist/pathologist.

We also observed that Methocult is a viable medium to support the growth and development of MGCs from the precursor stage onwards. Methocult was originally developed as an assay to ascertain the viability and multipotency of HSCs by supporting the growth of colony-forming units of various blood lineages.[5] In our study, Methocult demonstrated specific nutrient composition and a conducive microenvironment that enabled the formation of the intermediary adherent cells, which subsequently gave rise to MGC-like cells. This finding suggests that Methocult could be further explored as a method to generate MGCs *in vitro*.

We must also note that the findings of this study were based on the observations of abnormal HSC differentiation originating from donor cells with an unknown condition. Due to circumstances, we were hampered by the limited availability of starting materials and were not able to obtain additional samples for further analysis or to perform experimental replicates. The donor data pertaining to the sample provided to us was also limited and what was available was presented in this article.

CONCLUSION

This study presents the observation of MGC-like cell formation from umbilical cord blood mononuclear cell samples cultured in a methylcellulose medium. We postulate that the sample used for the aforementioned may be predisposed to infectious as it expressed high white blood cell counts and high percentages of distinct CD marker profiles of intermediary monocytic predisposition. Unlike conventional studies that rely on cytokine stimulation to generate MGCs, our findings suggest a potential alternative pathway for their development through an intermediary adherent epithelioid-like cell stage. This novel observation warrants further investigation to verify if the hypothesis of an alternative pathway of MGCs generation via linear-driven transitional phases influenced by prior physiological conditions is valid.

Acknowledgements: This study was made possible by funding from the UTAR Research Fund. Special appreciation also to Cryocord Sdn. Bhd. for their generous donation of crucial umbilical cord blood samples.

Conflicts of Interest: The authors declare they have no conflicts of interest.

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